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Citation for published version:

Wood, A, Lo, T-W, Zeitler, B, Pickle, CS, Ralston, EJ, Lee, AH, Amora, R, Miller, JC, Leung, E, Meng, X, Zhang, L, Rebar, EJ, Gregory, PD, Urnov, FD & Meyer, BJ 2011, 'Targeted genome editing across species using ZFNs and TALENs', *Science*, vol. 333, no. 6040, pp. 307. <https://doi.org/10.1126/science.1207773>

Digital Object Identifier (DOI):

[10.1126/science.1207773](https://doi.org/10.1126/science.1207773)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Science

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Published in final edited form as:

Science. 2011 July 15; 333(6040): 307. doi:10.1126/science.1207773.

Targeted Genome Editing Across Species Using ZFNs and TALENs

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Although entire genome sequences are available for numerous species, lack of reverse genetic tools has hindered cross-species comparisons of gene function. We demonstrate a widely applicable solution: targeted genome editing using site-specific nucleases. These engineered nucleases have fusions between the DNA cleavage domain of FokI and a custom-designed DNA binding domain: C₂H₂ zinc-finger motifs for zinc finger nucleases (ZFNs) (1) or truncated transcription activator-like effector (TALE) domains for efficient TALE nucleases (TALENs) (2). ZFNs and TALENs induce double-strand breaks at desired loci that can be repaired by error-prone non-homologous end-joining to yield small insertions and deletions (indels) at the break sites. We developed efficient procedures for targeted, heritable disruption of genes and cis-acting regulatory elements in the model nematode *Caenorhabditis elegans* and applied them to *C. briggsae*, a species diverged by 15 to 30 million years. Precisely targeted genome modifications had not been achieved in nematodes.

As proof of principle, we used ZFNs to disrupt a single-copy transgene expressing green fluorescent protein (GFP) in the *C. elegans* germline (Fig. 1A and table S1) (3). We overcame prior obstacles to attaining heritable, ZFN-induced mutations in worms (4) by injecting gonads with ZFN-encoding mRNAs carrying 5' and 3' untranslated regions favorable for germline translation (fig. S1). Progeny lacking green fluorescence carried indels at the target site, indicating heritable, targeted gene disruption (Fig. 1A and fig. S2A).

We next defined the time period after injection that yielded the highest proportion of mutant progeny (Fig. 1B and fig. S3, A to C). We targeted the endogenous gene *ben-1* with ZFNs, because *ben-1* mutants have an easily scorable phenotype: mobility on the paralysis-inducing drug benomyl (table S1). We found 5.1% of progeny from the peak 4-hour window to be mutants compared with 1.1% of total progeny. One in three injected animals produced mutants. A single animal could yield many mutant progeny, all with independent mutations (fig. S3C and table S2).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1207773/DC1

In *ben-1* mutants, we found no mutations at the top 39 off-target ZFN sites predicted by empirical specificity data (table S3, A and B). Moreover, sequence analysis of the entire *ben-1(y462)* mutant genome revealed only two indels: the ZFN-induced *ben-1* mutation and a deletion unlikely to have been caused by ZFN activity. The latter arose at a site unrelated to the ZFN site (fig. S4) and at a frequency consistent with spontaneous *C. elegans* indels (3).

To target mutagenesis of endogenous cis-regulatory elements, we devised methods to identify mutants by molecular genotyping, that is, without reliance on phenotype (fig. S3D) (3). We targeted *rex-1* on the X chromosome. *rex-1* recruits the dosage compensation complex (DCC) to reduce gene expression (5). Pivotal to DCC recruitment by *rex* sites is a 12-base pair (bp) motif called MEX that is enriched on X. We identified 32 *rex-1* mutations, including a partial deletion and a full duplication of the MEX motif (Fig. 1C and fig. S2B). Thus, ZFN-driven mutagenesis is sufficiently robust to obtain mutations without a priori knowledge of phenotype and is suitably precise to target small regulatory motifs.

Genome-editing protocols developed for ZFNs proved to be robustly portable to TALENs. DNA binding domains of TALEs have tandem repeat units (each 33 to 35 amino acids) in which the nucleotide binding preference is determined by two adjacent amino acids called the repeat variable diresidue (RVD) (6). By using previously described rules for altering DNA binding specificity of TALEs (6), we designed a pair of TALENs to target the same *ben-1* region as the ZFNs.

TALENs induced many *ben-1* mutations (Fig. 1D and fig. S2E) (3). Heterozygous mutants composed 3.5% of progeny in the peak window of mutagenesis defined for ZFNs. The spectrum of mutations was similar for ZFNs and TALENs (Fig. 1E and fig. S2, A to E).

To assess the nuclease delivery and mutant screening procedures across species, we applied them, without modification, to *C. briggsae*. By using ZFNs, we targeted the *C. briggsae* ortholog of the *C. elegans* gene *sdv-2*, which directs the DCC to assemble onto X and induces hermaphrodite sexual differentiation (5). Five independent *sdv-2* mutations were obtained (1.5% of progeny), thus establishing the efficacy of nuclease technology in *C. briggsae* (fig. S2C) (3). Our studies demonstrate a broadly useful strategy that brings targeted mutagenesis to nonmodel organisms and facilitates dissection of diverse biological processes.

Supplementary Material

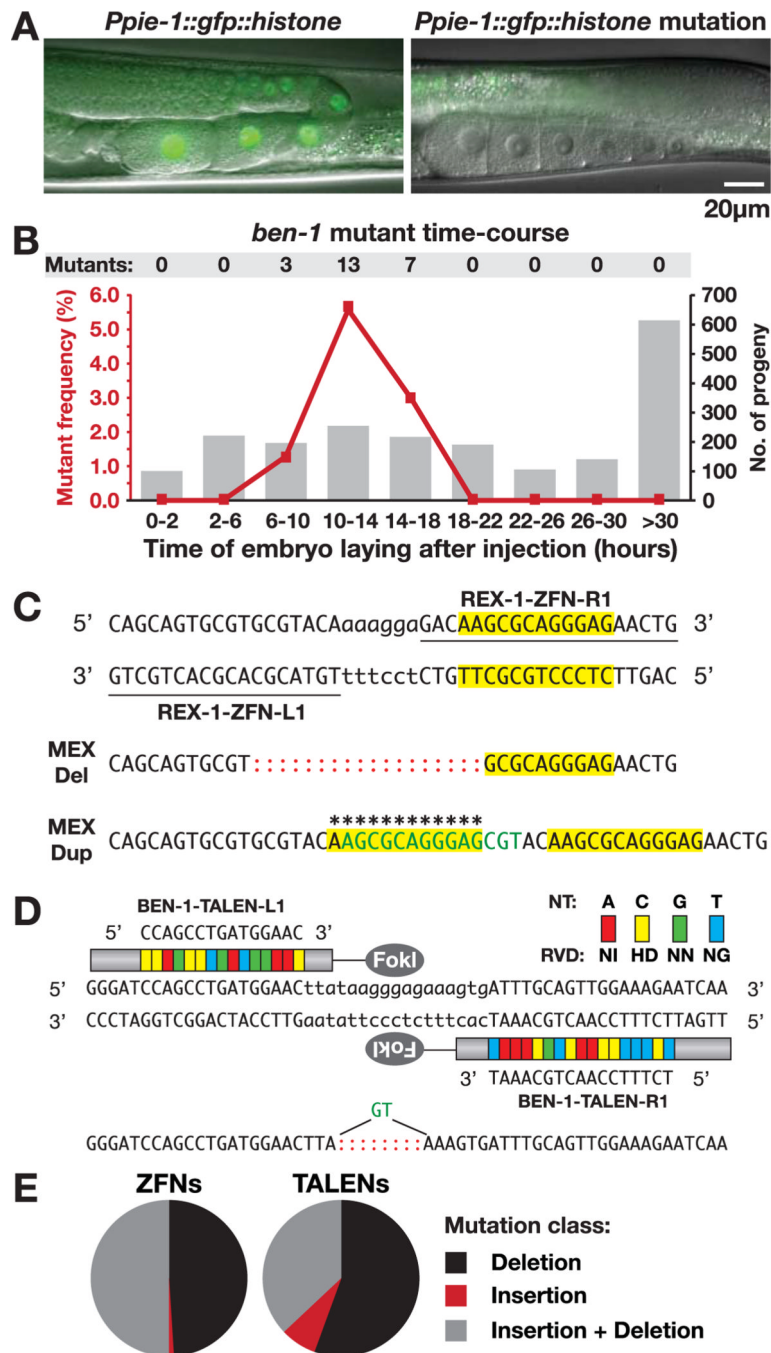
Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

A.J.W. is a Sir Henry Wellcome Post-Doctoral Fellow. NIH (GM30702) and HHMI funded these studies.

References and Notes

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**Fig. 1.**

Heritable, targeted nematode mutations induced by ZFNs and TALENs. (A) Hermaphrodite gonads with an intact (left) or ZFN-disrupted (right) *GFP::histone* transgene. Residual signal (right) is gut autofluorescence. (B) Frequency of *ben-1* mutants (left y axis, red line) from time periods after ZFN mRNA injection (x axis) relative to total progeny per period (right y axis, histogram). (C) ZFN-induced mutations in cis regulatory element *rex-1* cause partial deletion and full duplication (asterisks) of MEX motif (yellow). Represented are ZFN recognition sequences (underlined), FokI cleavage region (lowercase nucleotides), insertions (green), and deletions (red colons). (D) TALEN recognition sequences in *ben-1* and

TALEN-induced mutation. Each RVD (colored blocks) recognizes a nucleotide (NT) in the target site. (E) Proportion of each mutation class induced by ZFNs ($n=102$) and TALENs ($n=27$).